Enhancement of Progenitor Cells by Two-Step Centrifugation of Emulsified Lipoaspirates

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Abstract: BACKGROUND Adipose-derived stem cells, endothelial progenitor cells, and soluble factors jointly contribute to the regenerative effect of fat grafts. Nanofat grafting emulsifies the lipoaspirate and increases the progenitor cell yield. In the present study, the authors evaluated their extended nanofat grafting method that includes two additional centrifugation steps and results in a lipoaspirate of low volume that they termed "lipoconcentrate." Furthermore, the authors investigated the oily fractions after centrifugation for their regenerative potential. METHODS Lipoaspirates of 20 healthy patients were processed by emulsification and/or centrifugation. Six groups were created: native (not emulsified) fat, 1× centrifuged native fat, 2× centrifuged native fat, nanofat (emulsified), 1× centrifuged nanofat, and lipoconcentrate (i.e., 2× centrifuged nanofat). The oily phases after the centrifugation steps were collected. Progenitor cells and basic fibroblast growth factor, insulin-like growth factor 1, matrix metalloproteinase-9, platelet-derived growth factor-BB, and vascular endothelial growth factor-A levels were measured by flow cytometry and immunoassays. RESULTS Lipoconcentrate contained significantly higher numbers of adipose-derived stem cells and endothelial progenitor cells per gram compared with all other fractions. No difference of all five soluble factors between groups was found. The oily phases after centrifugation showed no or very few adipose-derived stem cells and endothelial progenitor cells, and no or very low levels of soluble factors. CONCLUSIONS Centrifugation of emulsified lipoaspirates increases the progenitor cell count in the lipoaspirate. The oily phase after centrifugation of lipoaspirates may be disposable because of the minuscule content of progenitor cells and soluble factors.

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Background: Adipose-derived stem cells, endothelial progenitor cells, and soluble factors jointly contribute to the regenerative effect of fat grafts. Nanofat grafting emulsifies the lipoaspirate and increases the progenitor cell yield. In the present study, the authors evaluated their extended nanofat grafting method that includes two additional centrifugation steps and results in a lipoaspirate of low volume that they termed "lipoconcentrate." Furthermore, the authors investigated the oily fractions after centrifugation for their regenerative potential.

Methods: Lipoaspirates of 20 healthy patients were processed by emulsification and/or centrifugation. Six groups were created: native (not emulsified) fat, 1× centrifuged native fat, 2× centrifuged native fat, nanofat (emulsified), 1× centrifuged nanofat, and lipoconcentrate (i.e., 2× centrifuged nanofat). The oily phases after the centrifugation steps were collected. Progenitor cells and basic fibroblast growth factor, insulin-like growth factor 1, matrix metalloproteinase-9, platelet-derived growth factor-BB, and vascular endothelial growth factor-A levels were measured by flow cytometry and immunoassays.

Results: Lipoconcentrate contained significantly higher numbers of adipose-derived stem cells and endothelial progenitor cells per gram compared with all other fractions. No difference of all five soluble factors between groups was found. The oily phases after centrifugation showed no or very few adipose-derived stem cells and endothelial progenitor cells, and no or very low levels of soluble factors.

Conclusions: Centrifugation of emulsified lipoaspirates increases the progenitor cell count in the lipoaspirate. The oily phase after centrifugation of lipoaspirates may be disposable because of the minuscule content of progenitor cells and soluble factors. (*Plast. Reconstr. Surg.* 142: 99, 2018.)

More than two decades have passed since Sydney Coleman revolutionized autologous fat grafting by his defined protocol.[1] Ever since, an exponentially rising number of publications have resulted in uncountable modifications of fat graft harvest, processing, and injection techniques, with the ultimate goal to increase retention and the regenerative potential of the grafted adipose tissue. Coleman's technique includes the harvest of adipose tissue by a blunt cannula followed by centrifugation of the lipoaspirate, which leads to three distinct layers: an upper oily layer, a middle purified fat layer, and a lower watery layer. After removal of the oily and watery layer, only the purified fat is routinely reinjected.

Earlier, we provided evidence that lipoaspirates contained significant amounts of basic fibroblast growth factor (bFGF), insulin-like growth

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factor 1 (IGF-1), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF).[2] The concentration of growth factors and other soluble factors in the transferred fat graft is of key importance, as they promote central angiogenic effects and increase the take rate of the fat graft.

Aside from soluble factors, progenitor cells such as adipose-derived stem cells and endothelial progenitor cells represent the second regenerative pillar of fat grafts. Adipose-derived stem cells are multipotent mesenchymal stem cells with a multilineage differentiation capacity, and endothelial progenitor cells are important progenitor cells that contribute to volume retention and neovascularization of fat grafts.[3,4] Numerous techniques have been proposed to enhance, in particular, the number of adipose-derived stem cells in the fat graft.[5] One simple maneuver to increase the progenitor cell content in fat grafts is the mechanical emulsification of adipose tissue, a technique called "nanofat grafting" suggested by Tonnard et al.[6] According to the authors, noncentrifuged lipoaspirates are shifted between two syringes through a Luer-Lok (Becton, Dickinson and Co., Franklin Lakes, N.J.) connector, leading to a disruption of adipocytes and concentration of progenitor cells.

In recent years, we have used a modification of Tonnard et al.'s original protocol by adding two centrifugation steps, ultimately resulting in a lipoaspirate with very little volume that we termed "lipoconcentrate." In the present study, we evaluated lipoconcentrate for its content of progenitor cells and important regenerative soluble factors. We compared lipoconcentrate with multiple control groups processed by various steps of emulsification and/or centrifugation to validate the necessity of each step of the lipoconcentrate preparation protocol.

Although we and other authors showed earlier that the watery fraction may have untoward effects and contains few growth factors, we also aimed to deliver scientific evidence for the removal of the oily fractions after centrifugation of lipoaspirates.[2,7] Thus, we further investigated the progenitor cell concentration and soluble factor levels in oily fractions after centrifugation.

PATIENTS AND METHODS

Patients and Adipose Tissue Harvest

Adipose tissue was harvested from the abdominal area of 20 healthy patients (six men and 14 women), with a mean age of 44.65 ± 3.73 years and a mean body mass index of 27.99 ± 1.23 kg/m^2, undergoing surgery at the Department of Plastic and Reconstructive Surgery, Hand Surgery–Burn Center at the University Hospital in Aachen. (**See Table, Supplemental Digital Content 1**, which shows the list of patients, ***http://links.lww.com/PRS/C804***.) After injection of a tumescent solution (sodium chloride 0.9% with epinephrine 1:800,000), lipoaspirates were harvested by means of a microfat grafting st'RIM cannula (Thiebaud Biomedical Devices, Margencel, France) connected to a 10-cc hand-held syringe as reported earlier.[8,9] Patients younger than 18 years or with a history of malignancies; autoimmune and cardiovascular diseases; or morbidities on the donor site such as scars, wound healing disorder, and dermatologic issues were excluded from the study. All operations were performed by a single plastic surgeon (N.P.). The use of human samples was approved by the regional ethics committee (EK163/07), and all experiments were conducted in compliance with the principles outlined in the Declaration of Helsinki.

Sample Preparation

Each of the 20 samples was divided into eight different fractions and further investigated as illustrated in Figure 1. All centrifugation steps were performed according to the Coleman protocol at 1200 *g* for 3 minutes.[10] Nanofat preparation (i.e., emulsification) included shifting of the lipoaspirate 30 times between two 10-ml syringes connected by a Luer-Lok female-to-female connector as described by Tonnard et al.[6] Samples were harvested, immediately processed, and transferred to the laboratory. The eight fractions included were as follows:

1. Native fat: Lipoaspirate after harvest without further manipulation.
2. 1× centrifuged native fat: The native fat was centrifuged. The resulting upper oily fraction and lower watery fraction were discarded, and the middle purified layer was collected.
3. Oily fraction: Upper oily layer after centrifugation of the native fat.
4. 2× centrifuged native fat: The 1× centrifuged native fat was centrifuged again. The resulting upper oily fraction and lower watery fraction were discarded, and the middle purified layer was collected.
5. Nanofat: Native fat underwent emulsification by the nanofat grafting protocol without centrifugation.

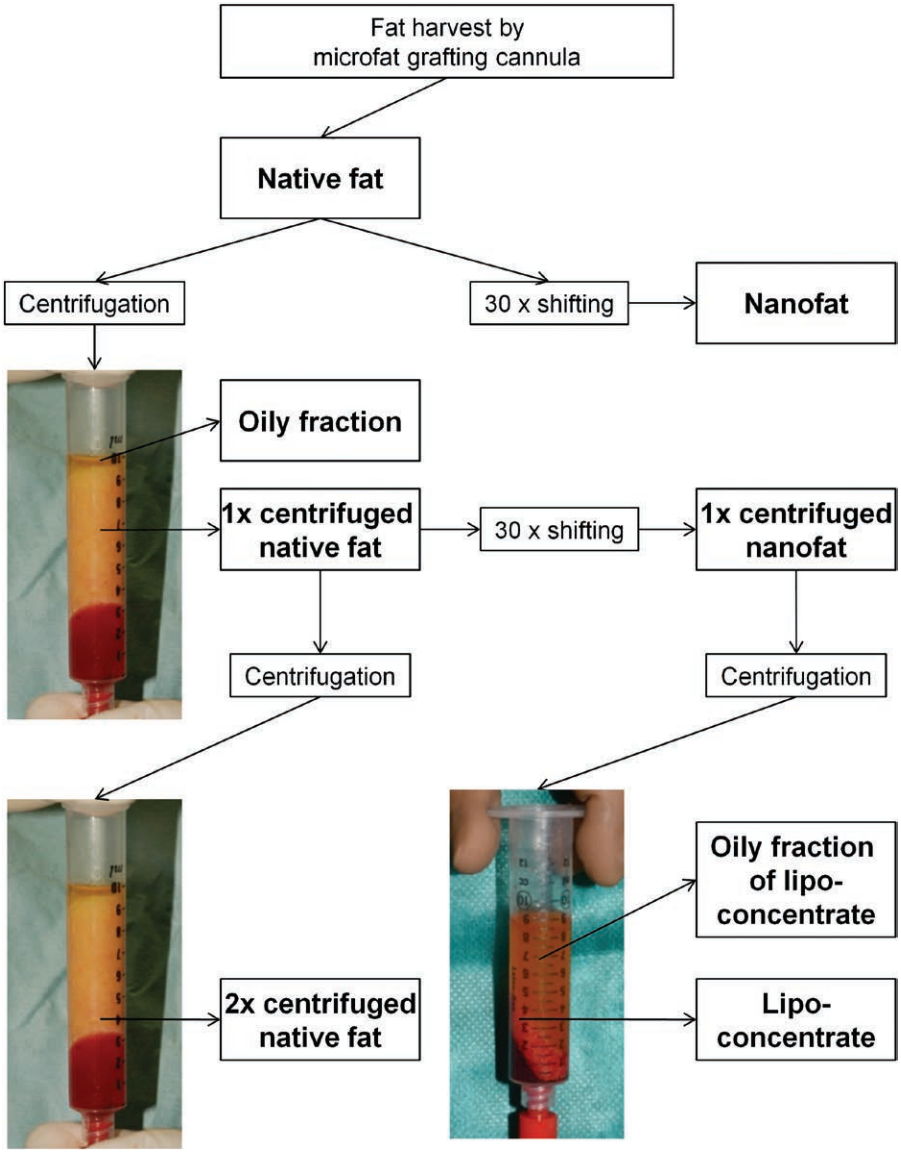

Fig. 1. Scheme for preparation of different fractions. For each patient, different fractions were collected as depicted. All centrifugation steps were performed according to the Coleman protocol at 1200 *g* for 3 minutes (Coleman SR. Facial recontouring with lipostructure. *Clin Plast Surg*. 1997;24:347–367). Emulsification included shifting of the lipoaspirate for 30 times between two 10-ml syringes connected by a Luer-Lok female-to-female connector (Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, Declercq H. Nanofat grafting: Basic research and clinical applications. *Plast Reconstr Surg*. 2013;132:1017–1026). All fat grafts were harvested with a microfat grafting cannula, resulting in native fat. Native fat is lipoaspirate after harvest without further manipulation. The native fat was either emulsified (i.e., nanofat) or centrifuged, which resulted in an upper oily (i.e., oily fraction), middle purified fat (i.e., 1× centrifuged native fat), and lower watery layer. The 1× centrifuged native fat was either centrifuged again or emulsified (i.e., 1× centrifuged nanofat). The middle fat layer after the second centrifugation of the 1× centrifuged native fat was called 2× centrifuged native fat. The middle layer after the second centrifugation of the 1× centrifuged nanofat was called lipoconcentrate. The upper oily layer was termed oily fraction of lipoconcentrate.

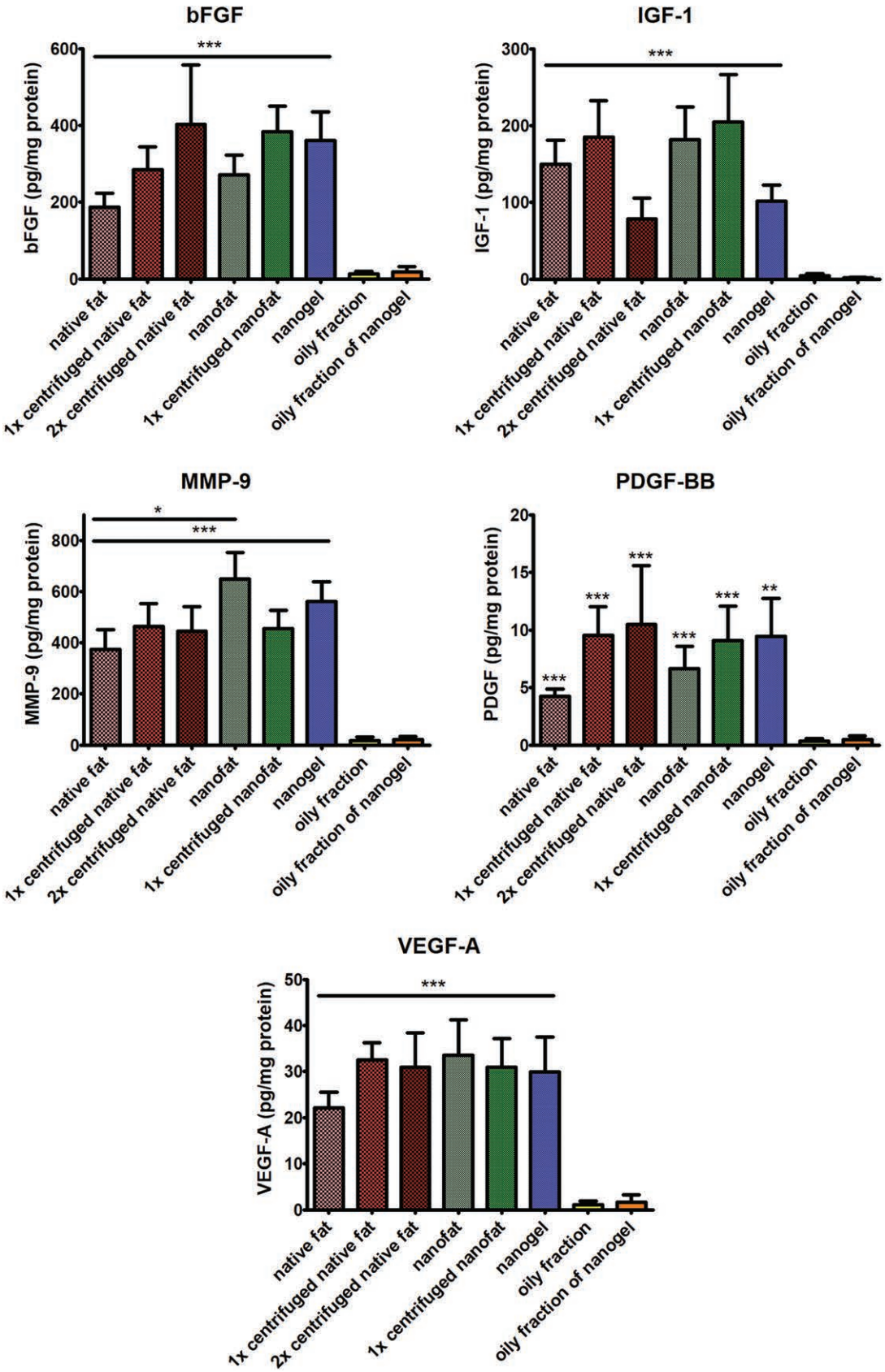

Fig. 2. Content of bFGF, IGF-1, MMP-9, PDGF-BB, and VEGF-A. The protein content of (*above*, *left*) bFGF, (*above*, *right*) IGF-1, (*center*, *left*) MMP-9, (*center*, *right*) PDGF-BB, and (*below*) VEGF-A was measured

6. 1× centrifuged nanofat: Native fat was first centrifuged. After discarding the upper oily fraction and lower watery fraction, the 1× centrifuged native fat underwent emulsification by the nanofat grafting protocol.
7. Lipoconcentrate: Native fat was first centrifuged. After discarding the upper oily fraction and lower watery fraction, the 1× centrifuged native fat underwent emulsification by the nanofat grafting protocol. The resulting 1× centrifuged nanofat was centrifuged again. The oily fraction of lipoconcentrate and watery fraction were removed, resulting in the purified liquefied fat layer that was termed lipoconcentrate.
8. Oily fraction of lipoconcentrate: Native fat was first centrifuged. After discarding the upper oily fraction and lower watery fraction, the 1× centrifuged native fat underwent emulsification by the nanofat grafting protocol. The resulting 1× centrifuged nanofat was centrifuged again. The resulting oily fraction was collected.

Measurement of bFGF, VEGF-A, PDGF-BB, IGF-1, and Matrix Metalloproteinase-9

Evaluation of soluble factors was performed as reported earlier.[11] Basic FGF, VEGF-A, PDGF-BB, and matrix metalloproteinase (MMP)-9 contents of the samples were analyzed by Multiplex magnetic bead Immunoassay (MilliporeSigma, Burlingon, Mass.). ProcartaPlex sets (eBioscience, Inc., San Diego, Calif.) were used according to the manufacturer's guidelines and measured on a Luminex 200 (Luminex Corp., Austin, Texas). The IGF-1 content was measured by an enzyme-linked immunosorbent assay Duo-Set (R&D Systems, Minneapolis, Minn.) on a FLUOstar OPTIMA microplate reader (BMG LABTECH, Aylesbury, United Kingdom) according to the manufacturer's guidelines.[11] The acquired content of the soluble factors were normalized to the protein level detected by the DC Protein Assay (Bio-Rad Laboratories, Inc., Hercules, Calif.).

Fig. 2. (*Continued*). in the eight fractions by Multiplex magnetic bead Immunoassay (bFGF, MMP-9, PDGF-BB, and VEGF-A) and enzyme-linked immunosorbent assay (IGF-1). The values were normalized to the total protein content. All data are presented as means ± SEM. Significant differences in soluble factor levels between the adipose tissue–containing fractions (native fat, 1× centrifuged native fat, 2× centrifuged native fat, nanofat, 1× centrifuged nanofat and lipoconcentrate) are indicated. Significant differences of adipose tissue–containing fractions when compared to both oily fractions (oily fraction, oily fraction of lipoconcentrate) are indicated as follows: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Stromal Vascular Fraction Cell Counting and Surface Marker Characterization by Flow Cytometry

Stromal vascular fraction cell counting was performed in a standard hemocytometer after lysis of erythrocytes with a red blood cell lysis buffer (eBioscience). Trypan blue viability stain (Thermo Fisher Scientific, Frankfurt am Main, Germany) was used to exclude dead cells. For fractions including adipose tissue (native fat, 1× centrifuged native fat, 2× centrifuged native fat, nanofat, 1× centrifuged nanofat, and lipoconcentrate), the stromal vascular fraction cell, adipose-derived stem cell, and endothelial progenitor cell counts were calculated as cells per gram starting tissue before collagenase digestion. For oily fractions, the stromal vascular fraction cell, adipose-derived stem cell, and endothelial progenitor cell counts were calculated as cells per gram solution.

Surface markers CD31, CD34, CD45, CD73, CD90, and CD105 were measured by flow cytometry as described earlier.[11] Samples including adipose tissue were digested with collagenase (collagenase type I; Worthington Biochemical Corp., Lakewood, N.J.), filtered, and centrifuged. The oily fraction and oily fraction of lipoconcentrate only were filtered without further collagenase digestion.

All antibodies were purchased from eBioscience and used for staining according to the manufacturer's instructions. Details of each antibody are found in Supplemental Digital Content 2. (**See Table, Supplemental Digital Content 2**, which shows the flow cytometry antibodies used, ***http://links.lww.com/PRS/C805***.) Stained cells were evaluated by flow cytometry on an LSR II cytometer (BD Bioscience, San Jose, Calif.).

Statistical Analysis

GraphPad Prism Version 5.03 (GraphPad Software, Inc., La Jolla, Calif.) was used for data analysis. One-way analysis of variance with repeated measures and with a post hoc multiple comparison test (Bonferroni) was used. All values are shown as means ± SEM. A value of $p < 0.05$ was considered significant.

RESULTS

Content of bFGF, IGF-1, PDGF-BB, VEGF-A, and MMP-9

There were no significant differences observed for bFGF, IGF-1, PDGF-BB, and VEGF-A between native fat and nanofat (Fig. 2).

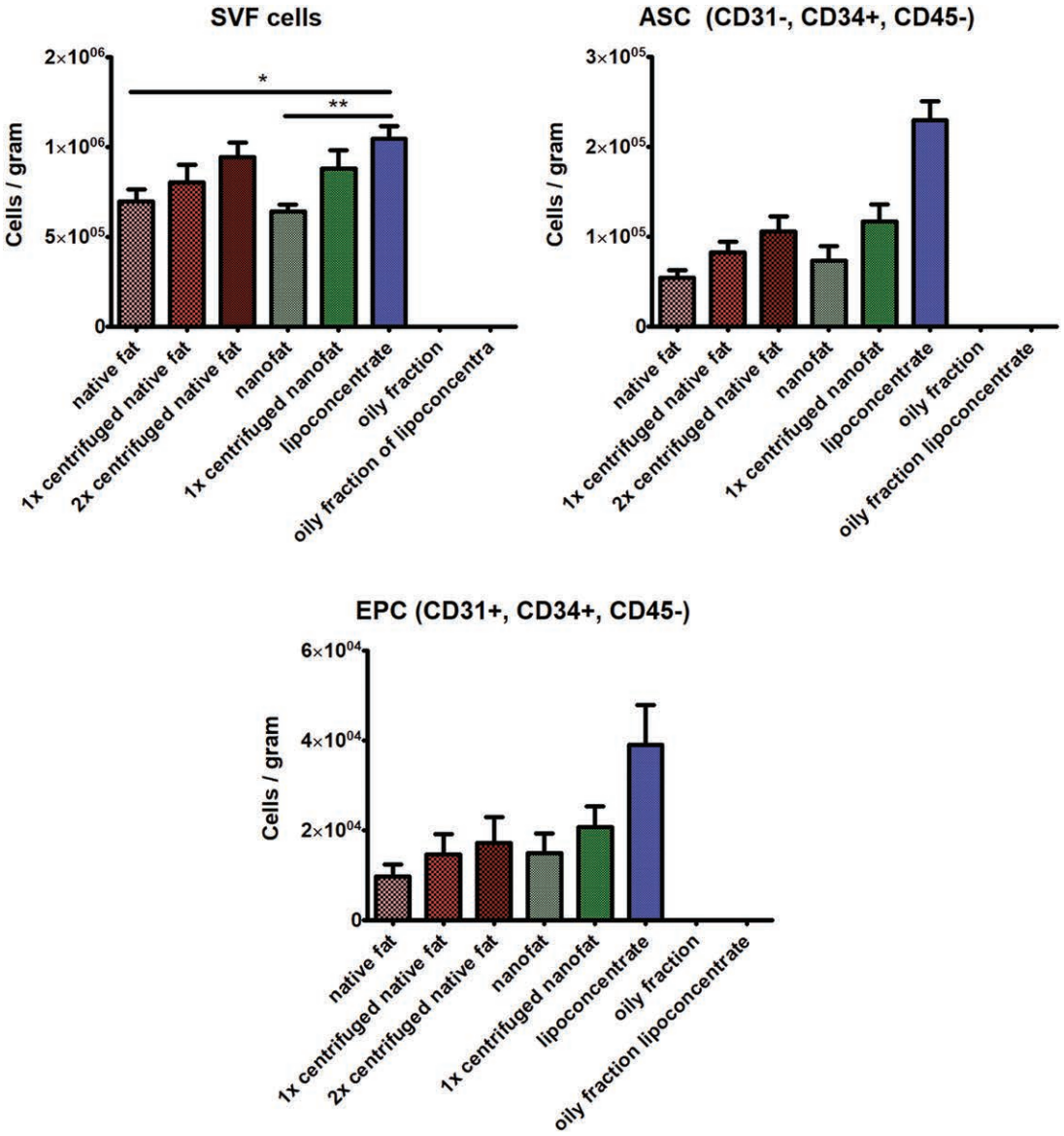

Fig. 3. Cell count and content of adipose-derived stem cells and endothelial progenitor cells. For each of the eight fractions, the cell count and the content of adipose-derived stem cells and endothelial progenitor cells were measured. (*Above*, *left*) Cell counting was performed in a standard hemocytometer after lysis of erythrocytes with red blood cell lysis buffer. A trypan blue viability stain was used to exclude dead cells. The cell count was calculated as cells per gram of tissue before collagenase digestion or per gram of solution. Significant differences are indicated as $*p < 0.05$ and $**p < 0.01$. (*Above*, *right*) Adipose-derived stem cells were phenotypically characterized as $CD31^-$, $CD34^+$, $CD45^-$ cells by flow cytometry and are presented as adipose-derived stem cell count per gram of tissue before collagenase digestion. Significant differences are illustrated in Table 1. (*Below*) Endothelial progenitor cells were phenotypically characterized as $CD31^+$, $CD34^+$, $CD45^-$ cells by flow cytometry and are presented as endothelial progenitor cell count per gram of tissue before collagenase digestion. Significant differences are illustrated in Table 2. All data are presented as means ± SEM. *SVF*, stromal vascular fraction; *ASC*, adipose-derived stem cells; *EPC*, endothelial progenitor cells.

Centrifugation also did not have significant effects on the levels of bFGF, IGF-1, PDGF-BB, and VEGF-A. MMP-9 levels were significantly higher in nanofat (648.0 ± 105.0 pg/mg; $p < 0.05$) compared with native fat (374.8 ± 76.49 pg/mg; $p < 0.05$). The levels of all five soluble factors were significantly lower in both oily fractions than in the other fractions ($p < 0.01$ or $p < 0.001$). In most oily samples, the protein levels were even below the detection limit.

Stromal Vascular Fraction Cell Count

Among samples including adipose tissue, lipoconcentrate showed the highest concentration of cells after collagenase digestion (Fig. 3, *above, left* and Table 1). The number of stromal vascular fraction cells in lipoconcentrate ($1.045 \pm 0.071 \times 10^6$ cells/g) was significantly higher than in nanofat ($0.642 \pm 0.036 \times 10^6$ cells/g; $p < 0.01$) and native fat ($0.698 \pm 0.065 \times 10^6$ cells/g; $p < 0.05$). The oily fraction of lipoconcentrate was markedly larger than the oily fraction in general. In both oily fractions, the stromal vascular fraction cell number fell below the detection limit.

Adipose-Derived Stem Cell Count

Adipose-derived stem cells were defined as $CD31^-$ (endothelial marker), $CD34^+$ (stem cell marker), $CD45^-$ (hematopoietic marker) cells (Fig. 3, *above, right*).[12] Lipoconcentrate showed a significantly higher adipose-derived stem cell concentration ($0.229 \pm 0.021 \times 10^6$ cells/g) compared with any other fraction ($p < 0.01$ or $p < 0.001$) (Table 1) and more than two-fold more adipose-derived stem cells compared with native fat ($0.054 \pm 0.008 \times 10^6$ cells/g). The oily fractions both showed no or small numbers of adipose-derived stem cells.

Endothelial Progenitor Cell Count

A similar trend was seen for $CD31^+$, $CD34^+$, $CD45^-$ endothelial progenitor cells (Fig. 3, *below*). Lipoconcentrate showed by far the highest endothelial progenitor cell concentrations (Table 2). A significant difference was found between lipoconcentrate ($0.389 \pm 0.089 \times 10^5$ cells/g) and native fat ($0.096 \pm 0.027 \times 10^5$ cells/g; $p < 0.01$), 1× centrifuged native fat ($0.145 \pm 0.045 \times 10^5$ cells/g; $p < 0.05$), and 2× centrifuged native fat ($0.171 \pm 0.058 \times 10^5$ cells/g; $p < 0.05$). Both oily fractions showed very few endothelial progenitor cells.

Relative Expression of CD73, CD90, and CD105 on Adipose-Derived Stem Cells and Endothelial Progenitor Cells

Next, the relative expression of the mesenchymal markers CD73, CD90, and CD105 on the adipose-derived stem cells was measured (Fig. 4, *left*).[13] However, no significant differences were seen in the relative ratios of $CD73^+$, $CD90^+$, $CD105^+$, and $CD73^+/CD90^+/CD105^+$ adipose-derived stem cells. We refrained from characterizing CD73, CD90, and CD105 on adipose-derived stem cells of the oily fractions as the number of cells was too small (data not shown).

Table 1. Significant Differences in Adipose-Derived Stem Cell Content

	1× Centrifuged Native Fat	2× Centrifuged Native Fat	Nanofat	1× Centrifuged Nanofat	Lipoconcentrate	Oily Fraction	Oily Fraction of Nanogel
Native fat	NS	NS	NS	NS	***	NS	NS
1× centrifuged native fat		NS	NS	NS	***	*	NS
2× centrifuged native fat			NS	NS	***	**	*
Nanofat				NS	***	**	*
1× centrifuged nanofat					**	***	***
Lipoconcentrate						***	***
Oily fraction							NS

NS, not significant.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

Table 2. Significant Differences in Endothelial Progenitor Cell Content

	1× Centrifuged Native Fat	2× Centrifuged Native Fat	Nanofat	1× Centrifuged Nanofat	Lipoconcentrate	Oily Fraction	Oily Fraction of Nanogel
Native fat	NS	NS	NS	NS	**	NS	NS
1× centrifuged native fat		NS	NS	NS	*	*	*
2× centrifuged native fat			NS	NS	*	*	*
Nanofat				NS	NS	**	**
1× centrifuged nanofat					NS	***	***
Lipoconcentrate						***	***
Oily fraction							NS

NS, not significant.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

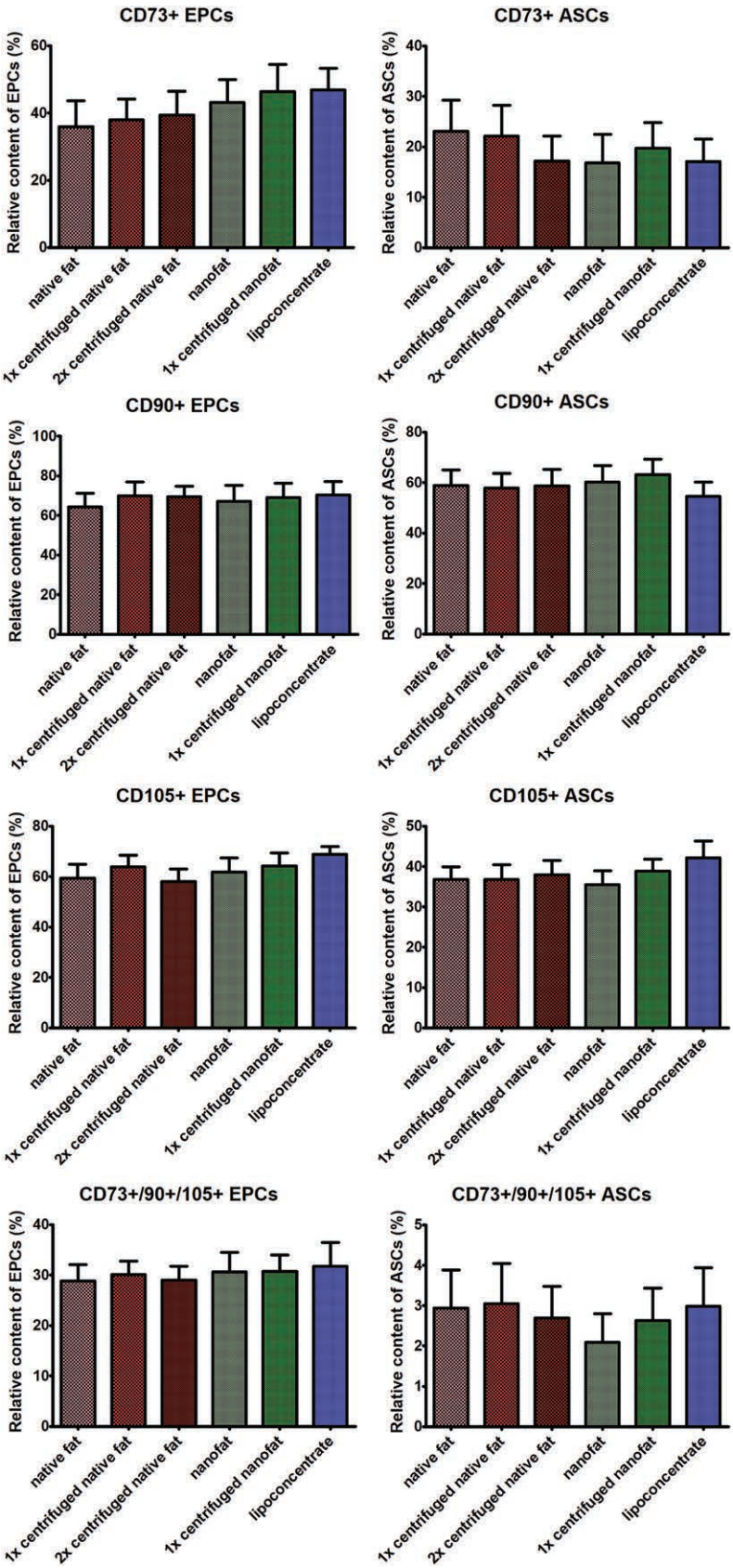

Fig. 4. CD73, CD90, and CD105 expression on adipose-derived stem cells (*ASCs*) and endothelial progenitor cells (*EPCs*). Relative expression

Relative CD73, CD90, and CD105 expression on endothelial progenitor cells showed no significant differences among the fractions (Fig. 4, *right*). We again refrained from characterizing CD73, CD90, and CD105 on endothelial progenitor cells of the oily fractions, as their number was too low (data not shown).

DISCUSSION

In the present study, we propose a modification of Tonnard et al.'s original nanofat grafting protocol by double centrifugation eliciting an even more liquid lipoaspirate called lipoconcentrate. Some authors regard centrifugation to be a superfluous method and rather advocate more conservative processing techniques such as decantation, washing, or even immediate reinjection without any processing method applied at all.[14] In our view, centrifugation has its raison d'être, as it is a safe, simple, and effective measure to separate the lipoaspirate from fractions with few progenitor cells and soluble factors.[2,15] Although we showed earlier that the watery layer of lipoaspirates after centrifugation contained significantly lower levels of growth factors compared with purified fat,[2] in the current study, we also found significantly lower levels of soluble factors and progenitor cells in the oily fractions. In other words, from the perspective of regenerative potential, we do not see any rationale to keep the watery or the oily fraction, as they occupy unnecessary volume devoid of regenerative cells and soluble factors. Also, superfluous oil may be a reason for volume loss of the fat graft and oil cysts.[16]

We have found increasing numbers of stromal vascular fraction cells, adipose-derived stem cells, and endothelial progenitor cells per gram of tissue with each centrifugation step in the native and the emulsified fractions. The lipoconcentrate had by far the highest adipose-derived stem cell and endothelial progenitor cell counts. We assume two reasons for this observation. First, the elimination of oily and watery layers by centrifugation leads to a relative increase in cells per gram of starting material. The particularly voluminous oily layer after the second centrifugation step of the emulsified fat could explain the remarkable increase of cells per gram of tissue between 1× centrifuged nanofat and lipoconcentrate. Mashiko et al. observed that emulsification of lipoaspirates led to damage of adipocytes and an increase of adipose-derived stem cells and endothelial cells compared with centrifuged and squeezed lipoaspirates.[17] They also found few adipose-derived stem cells in the residual fluid after filtration of emulsified fat, which is in line with our observations. The second explanation is a potential phenotypic change of stromal vascular fraction cell fractions by mechanical forces as demonstrated by Banyard et al., who found altered marker expression by nanofat processing.[18] The fact that lipoconcentrate shows higher progenitor cell counts than 1× centrifuged nanofat and 1× and 2× centrifuged native fat indicates that only a combination of emulsification and two centrifugation steps—which is the key of our technique—results in the significant increase in adipose-derived stem cell and endothelial progenitor cell enrichment found in lipoconcentrate.

We chose two separate centrifugation steps for the preparation of lipoconcentrate for the following reasons: the first centrifugation step immediately after harvest clears the fat graft of potentially harmful other fractions, including the tumescent solution, and allows emulsification of the relevant adipose tissue fraction. The second centrifugation step then eliminates the remnants of the lysed cells. A longer first centrifugation period may not address the second issue and vice versa. We did refrain from increasing centrifugation force, as the settings proposed by Coleman are most widely used, and several studies suggest 1200 *g* as appropriate, with higher forces potentially being harmful.[6,7]

Progenitor cell enrichment generally is possible by either mechanical or enzymatic methods. Enzymatic methods rely on enzymatic digestion of the collagen in the extracellular matrix amended by centrifugation, filtering, and washing.[19] There is little contention that enzymatic methods yield higher progenitor cell numbers. At the same time, however, it entails regulatory issues and risk of contamination and, above all, requires a fully equipped laboratory facility. To bypass the aforementioned issues, semiautomated and fully automated systems were developed.[20,21] Major drawback to the commercially available systems are their high acquisition and maintenance costs, inconsistent cellular yield and—depending on the device—long preparation time.[19] Tonnard et al.'s original nanofat-graft technique, Mashiko et al.'s technique of nanofat preparation of centrifuged fat, and now our lipoconcentrate preparation

Fig. 4. (*Continued*). of the mesenchymal markers CD73, CD90, and CD105 on (*left*) adipose-derived stem cells ($CD31^-$, $CD34^+$, $CD45^-$) and (*right*) endothelial progenitor cells ($CD31^+$, $CD34^+$, $CD45^-$) on the cell surface was measured by flow cytometry. All data are presented as means ± SEM. No statistical differences between fractions were observed.

protocol therefore represent attractive alternatives, as they only require a centrifuge, standard syringes, and a female-to-female Luer-Lok connector. Moreover, our protocol can be performed in less than 10 minutes in the operating room.

There also is an ongoing debate on the most efficient fat graft processing technique, including washing, gravity separation, filtration, and centrifugation. Although the literature is not conclusive, centrifugation as part of Sydney Coleman's established protocol is widely used, and its efficacy is supported by several experimental studies.[9] Clinical studies have also shown increased longevity and better aesthetic outcome of centrifuged fat compared with noncentrifuged fat[10] and no nodule formulation of centrifuged fat compared with filtered fat.[11] At the same time, aesthetic results of centrifuged fat appear to be comparable to those of costly automated fat graft processing devices.[12] The true value of lipoconcentrate compared with commercially available systems, enzymatic digestion, and other processing techniques, however, is subject to future research.

We characterized adipose-derived stem cells and endothelial progenitor cells by their surface CD34 expression. Besides CD34, adipose-derived stem cells and endothelial progenitor cells also express other central stem cell markers, of which CD73, CD90, and CD105 were selected as minimum criteria for multipotent mesenchymal stem cells by the International Society for Cellular Therapy[13] and are frequently used to characterize cells of the stromal vascular fraction.[12,22] However, our results indicate that processing techniques such as centrifugation and emulsification generally did not have any influence on CD73, CD90, and CD105 expression of endothelial progenitor cells and adipose-derived stem cells.

Fat grafts contain and secrete copious amounts of soluble factors that orchestrate tissue homeostasis.[23] We have chosen a panel of four growth factors and the enzyme MMP-9, as their regenerative effect is well documented in the literature. In fact, several in vivo studies have shown improved fat graft retention and vascularization by supplementation of bFGF,[24] IGF-1,[25] PDGF,[26] and VEGF.[27] MMP-9 is a well-studied enzyme for extracellular matrix degradation and is involved in adipose tissue differentiation and wound remodeling.[28,29] In an earlier publication, we have shown that the content of growth factors is significantly lower in the watery phase compared with the purified fat.[2] Lipoconcentrate contained substantial concentrations of the aforementioned soluble factors. Nonetheless, no statistical difference between lipoconcentrate and the other fat tissue–containing fractions was present, suggesting that the regenerative property of lipoconcentrate may primarily originate from progenitor cells.

From a clinical point of view, the lipoconcentrate may be particularly useful for the treatment of areas where a regenerative effect of fat graft by means of adipose-derived stem cells and endothelial progenitor cells is desired more than a volume expansion. Indications include the rejuvenation of the perioral or buccal area or the treatment of scars, where progenitor cells contribute to restoration of the epidermis and dermis[1,2] and improve scar appearance.[3] The lipoconcentrate also may be useful for the treatment of chronic wounds with malperfusion. In these wounds, limited vascularization prevents voluminous fat grafts from promptly receiving blood supply from the surrounding tissue and thus results in fat necrosis.[4] In this context, transplanted endothelial progenitor cells in particular may assist in neovascularization.[5]

CONCLUSIONS

In the present work, we introduce a new method of fat graft processing that includes centrifugation of the harvested fat followed by emulsification and a second centrifugation step resulting in a graft termed lipoconcentrate. The lipoconcentrate contains significantly higher levels of adipose-derived stem cells and endothelial progenitor cells but unaltered soluble factor levels compared with other fractions. We assume that emulsification leads to a disruption of adipocytes that are eliminated by centrifugation, ultimately concentrating progenitor cells per gram of tissue. Lipoconcentrates may be particularly useful for applications where the tissue regeneration is desired more than a volumetric effect. Our work is primarily of a descriptive nature, and translation of our experiments in animal studies is necessary to draw a conclusion on the clinical relevance of our results.

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